Assessment of Cell Viability by Flow Cytometry

After staining cells, but before running on flow cytometer, add the following amount/concentration of the appropriate cell viability dye to discriminate between live/dead cells.
The principle is the same for most of these dyes: these dyes enter the cells if the cell membrane is compromised; as such, cells that stain with these dyes are dead, and cells that do not stain are considered live.

Propidium Iodide (PI):

This fluoresces in both the FL2 and the FL3 channel. As such, one has to use 2 channels to get information only from one. One can use an FL3 dye and collect PI fluorescence from FL2, but compensation is usually very high/impossible. Take home message: use only PI in FL2 and FL3 is using this dye.

Stock: 50ug/uL in ethanol/PBS
Add: 1uL per 100uL of media in tube.
Caution: PI is carcinogenic. Wear gloves.

7-Aminoactinomycin D (7 AAD)

This fluoresces maximally at approximately 646nM (FL3 channel).
Compensation is usually low, and must be done out of FL2 and FL4 is appropriate.
This only fluoresces in FL3, which differs from PI (PI offers no advantage for live/dead discrimination over 7AAD that I know of).

Stock: 1mg/mL in MeOH
Add: 1uL per 100uL of media in tube
Caution: Potential carcinogen, absorbed through skin. Wear gloves.

To-Pro3

This dye is excited around 630 and emits at about 660 – so, you need a HeNe light source to excite it, and it fluoresces in the FL4 channel of the FACSCalibur (just like APC).
Because it fluoresces in FL4, compensation – usually of a low percentage – is necessary out of FL3 if appropriate.

Stock: 1mM in DMSO
Add: 1uL per 500uL to 1mL of solution
Caution: This reagent is not toxic, but it is dissolved in DMSO.

Ethidium Monoazide (EMA): Protocol is different than other dyes

This dye fluoresces in the FL3 channel – approximately 635nM. I it is like 7AAD in this way. However, EMA is unique in that it irreversibly binds DNA and can be detected after fixation for long periods of time (days), so that it is the ideal dye for use if you are dealing with biohazard samples.

EMA is unique because it irreversibly crosslinks DNA. The protocol for EMA is the following:

Add EMA (final concentration of 5ug/mL) to cells (1.0x10^6) in tubes. Titrate dye for higher/lower cell number.

Let cells sit (on ice of possible) in the light (yes, IN the light) for 20-30 minutes.

Wash cells once with PBS.

Stain for other markers on ice in dark as you would normally.

Cells can now be run on cytometer unfixed, or if they need to be fixed, they should be fixed in 1% paraformaldehyde for at least 10 minutes before running.

Stock: 50ug/mL in EtOH

Add: 5ug/mL per 1x10^6 cells

Caution: May be carcinogenic. Wear gloves.